Early-season growth responses of corn hybrids and inbred lines to a commercial seed treatment.

By

Rose V. Vagedes

Ohio State University

Horticulture and Crop Science Department

Columbus, OH 43210

Research Advisor

Alexander Lindsey, Ohio State University

Core Ideas

- Measured early-season growth responses of commercial hybrids and recombinant inbred lines to seed treatment of clothianidin +*B.firmus*.
- Most early-season growth parameters were greater when seeds were treated, aside from root porosity
- The seed treatment used in this study may accelerate early-season development, but more research is needed to assess its role in improving abiotic stress tolerance.

Abstract

Annual rainfall in the Midwest has increased up to 10% since the early 20th century, while approximately 20-25% of the global corn crops have experienced yearly drought. Climate variability has increased demand for research on how management practices impact crop growth. In 2019, an open-air pot study was conducted in central Ohio to assess how a commercial seed treatment (Poncho 1250 + VOTiVO) would impact early-season above- and below-growth of corn (*Zea mays L.)*. Two recombinant inbred lines and two commercial hybrids were selected, with each corn line treated with clothianidin + *Bacillus firmus* I-1582 (Poncho 1250 + VOTiVO) or untreated prior to planting. Seed treatment had a positive effect on growth staging, plant height, and root surface area at termination. However, root porosity was unaffected by seed treatment aside from one commercial hybrid where porosity decreased with seed treatment. Application of Poncho 1250 + VOTiVO improved early-season growth of seedlings, but more research is needed to understand its impact on improving crop tolerance to early-season abiotic stresses.

Abbreviations

CH1, commercial hybrid 1; CH2, commercial hybrid 2; T, treated; UT, untreated; RCA, root cortical aerenchyma; RIL, recombinant inbreed line

Introduction

Climate Extremes

Throughout the growing season, corn encounters numerous abiotic stress factors, such as drought and flooding, which inhibit crop production. It is estimated by Heisey et al. (1999), that every year approximately 20-25% of the worlds corn production is affected by drought. Corn's susceptibility to drought is much greater compared to other cereals, which cause major yield loss in large corn producing countries, like the United States (Heisey et al. 1999). Since the 1970s the United States has also been experiencing a notable increase in excessive wetness, particularly, regions of southern Mississippi River valley, Southwest, Midwest, and Great Lakes (Karl et al., 1996; Easterling et al., 2010). According to Easterling et al. (2010), these regions have experienced an increase in total annual precipitation between 5% and 10% since early the 20th century. The United States has also experienced an increase in single day rainfall events that exceed 50.2 mm by 2% since the 1910s (Karl et al, 1996). The increased frequency of heavy rainfall events is predicted to increase by as much as 20% in the Midwest from 2071-2099 as well (Melillo et el., 2014). Regardless of whether the stress is from excessive rainfall or excessive drought, yield reductions can be similar in magnitude (34% or 37%, respectively) compared to yield trend averages (Li et al., 2019).

Root Cortical Aerenchyma

One mechanism that plants use to tolerate the stress of flooding and drought is through the formation of root cortical aerenchyma (RCA) (Drew et al 2000; Zhu et al 2010). RCA are large intercellular spaces formed in the root cortex to assist with the respiration of oxygen in hypoxic environments (Drew et al., 2000). When hypoxia caused by flooding is detected, corn will begin lysigenous aerenchyma formation by signaling programmed cell death less than a centimeter behind the root apical meristem (Drew et al 2000). The levels of RCA formation in corn roots can vary from one genotype to the other (Zhu et al 2010). In a study conducted by Zhu et al. (2010), recombinant inbreed lines (RIL) of corn with high RCA exhibited greater drought tolerance compared to RILs with lower RCA production. The authors suggest high RCA producing lines are capable of deeper root exploration, higher shoot biomass, and higher yields compared to the genotypes with low RCA due to reduced energy losses from root cortical cells (Zhu et al, 2010).

Poncho 1250 + VOTiVO seed treatment

Covering more than 16 million hectares, Poncho/VOTiVO is the highest selling seed treatment in the United States (AGDAILY Staff, 2017). This treatment is beneficial for its 1,000-fold bacterial load reduction per acre, broad-spectrum protection from nematodes and insects, improved root health, and the yield advantages (Wilson et al, 2013). The systemic active ingredient of ‘Poncho’, clothianidin, also provides broad-spectrum insect control for above and below ground plant structures (Poncho VOTIVO Seed Treatment, 2019). The ‘VOTiVO’ component of the seed treatment, *B. firmus*, can help reduce nematode feeding in crop roots (Wilson et al, 2013). It is this component of the seed treatment that promotes healthy plant and root growth as well (Poncho VOTIVO Seed Treatment 2019).

In light of recent trail data, it has been suggested that this may also increase, RCA formation (R. Tocco, personal communication, 15 Nov. 2017). The objective of this study was to examine the effect on early-season growth, including RCA development in both commercial hybrids and RILs in response to Poncho 1250 + VOTiVO seed treatment.

Material and Methods

This study was conducted in 2019 from May-October in an open-air greenhouse located at the Ohio State University in Columbus, OH (40.0022˚N, 83.0285˚W, 275m elevation). A randomized complete block design with a full factorial of corn line and seed treatment was conducted with four replications of treatment per run. A total of four runs of the experiment were conducted. Four corn lines were evaluated in total with two being RILs (M0331 and M0364) that had previously been identified as low or high RCA formers, respectively, (Zhu et al., 2010), and two being modern commercially available hybrids (CH1 and CH2) of which CH2 had been identified in company literature as having superior drought tolerance.

The seed treatment factors were untreated or treatment with a blend of Poncho/VOTiVO + Poncho 600 for a total application rate of (1.25 mg a.i. kernel^{-1} clothianidin + 0.045 mg a.i. kernel^{-1} *B. firmus* I-1582), which contains the equivalent clothianidin rate of Poncho 1250 + VOTiVO, a common use rate for corn production (AgVenture, 2011). Each corn line had 300 seeds treated three days prior to the start of the first run. Excess seeds were stored at 10^{o}C between runs. Pots (11.3-L volume) were filled with an amended field soil (Premium Blend Soil, Price Farms Organics) with 17 g kg^{-1} organic matter, a pH of 7.8, a cation exchange capacity of 27.5 cmol(+) kg^{-1}. For each pot, two seeds were planted at a depth of 5 cm. For the third and fourth run, emergence date for each pot was recorded. Once all plants reached a growth stage between VE-V1 (staging method described by Abendroth et al., 2011), pots were thinned to one plant. Pots were watered once per week using a soluble fertilizer pack (Miracle-Gro Watering Can Singles: All-Purpose Water-Soluble Plant Food, Scotts, Marysville, OH). Natural rainfall was monitored and supplemental irrigation was added as needed to ensure water stress was not incurred.

Plants were harvested once they achieved a growth stage of V4-V6. On the day of harvest, plant height, leaf greenness, and plant growth stage (V staging method) were recorded. Plant height was taken from the soil line to the tip of the longest leaf. Leaf greenness was collected by using a SPAD meter placed in the center of the youngest collared leaf. Once plants were removed from the pots, the roots were gently washed in water. The above- and below-ground biomass was separated at the node located just above the root system. The above-ground biomass was placed in a drier set at 60^{o}C for at least five days then weighed. Root porosity was measured as a proxy of RCA using procedures outlined in Thomson et al. (1990). The below-ground fresh biomass was weighed before being weighed in a basket fully submerged under water suspended below the scale. This was done to calculate the volume of the root system using Eq. 1:

Equation 1: $$V_{root} = \frac{RW_{air} - RW_{water}}{D_{water}}$$

where V_{root} =volume of root, RW_{air} =weight of root in air, RW_{water} =weight of root in water, and D_{water} = density of water. The roots were transferred into in a vacuum infiltration chamber and exposed to three 20 s intervals of approximately 185 kPa. The roots were transferred back in the suspended basket and weighed again. This was completed to calculate the volume of gas in the root system using Eq. 2:

Equation 2: $$V_{gas\,root} = \frac{RW_{vacuum} - RW_{water}}{D_{water}}$$

where $V_{gas\,root}$ = volume of gas in roots, RW_{vacuum} = weight of vacuumed root, RW_{water} =weight of root in water, and D_{water} = density of water. Finally, percent porosity was calculated using Equation 3:

Equation 3: $$\%\,Porosity = \frac{V_{gas\,root}(100)}{V_{root}}$$

where $V_{gas\,root}$ = volume of gas in roots, and V_{root} =volume of root.

Following this procedure, roots were stored in plastic bags at 10^{o}C until they were scanned and analyzed for length and area using WhinRhizo within one week of harvest. Once scanned, roots were placed in labeled paper bag and dried at 60^{o}C for approximately 5 days then weighed.

All data were subjected to analysis of variance using the GLIMMIX procedure in SAS 9.4. The fixed effects for the model consisted of corn line, seed treatment, and their interaction, with run and replication nested within run as the random effects. When the global F-test was significant (alpha=0.1), means were separated using the LSMEANS statement.

Results

Root Porosity

While differences in corn lines were evident for most measured parameters, the absence of interactions between corn line and seed treatment suggests similar responses were observed across lines for all parameters except root porosity (P=0.028; Figure 1). Most lines were non-responsive to seed treatment for root porosity, but the root porosity for CH1 decreased with seed treatment.

Plant Growth and Development

Average growth stage at the time of harvest across corn lines was 4% greater when seeds were treated prior to planting compared to non-treated (Table 1). Additionally, plants that received the seed treatment were 7% taller than those that were non-treated. While differences in root length impacted by seed treatment were not significant the root surface area was increased when seed were treated by 7% compared to the non-treated. However, total root and shoot biomass were not affected by inclusion of the seed treatment. Additionally, seed treatment did not affect leaf SPAD values. For the third and fourth run, seed treatment also did not affect emergence date (data not shown).

Tables and Figures

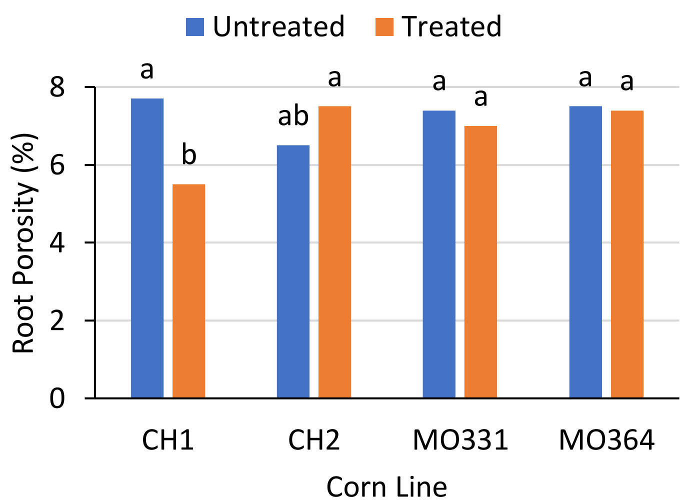

Figure 1: Percent root porosity for each corn line as a result of treatment with clothianidin+*B. firmus* or untreated. Data is presented across runs. Different letters denote significant differences between treatment means for the corn line x treatment interaction (alpha= 0.1).

Table 1: Growth Stage, height, shoot biomass, leaf greenness (SPAD), root length, area, and root biomass at termination as impacted by seed treatment across corn lines. Data presented across runs. Different letters denote significant differences between treatment means (alpha=0.1).

Seed Treatment	**Stage**	**Height**	**Shoot Biomass**	**SPAD**	**Root Length**	**Root Area**	**Root Biomass**
		cm	**g**		**cm**	**cm^2**	**G**
Treated	5.0a	55.1a	1.41a	33.1a	1412.3a	305.2a	0.59a
Untreated	4.8b	51.5b	1.22a	33.0a	1322.5a	285.9b	0.52a
P-values	0.031	0.058	0.242	0.911	0.145	0.069	0.150

Discussion

Corn and many other cereal crops are known to produce lysigenous aerenchyma tissue when programmed cell death is triggered in the root cortex by abiotic factors that are unfavorable to root respiration (Pujol, 2018). When corn cultivars were grown in sand, aerenchyma was found in both the flooded and controlled (well-drained) treatments but the ratio of aerenchyma formation to root cortex was significantly greater under flooded conditions than those grown in well-drained soils (Lenochova, 2009). It is understood that under non-stressed conditions, maize can form some aerenchyma tissue, but levels increase with increasing ethylene synthesis (Lenochova, 2009). Lack of imposed nutrient or water stress may have contributed to the root porosity response to seed treatment. Different results may have been observed if environmental stresses were present. Additionally, using the blended seed treatment material in this trial reduced the concentration of *B. firmus* as compared to using Poncho/VOTiVO alone (0.50 mg ai kernel^{-1} clothianidin + 0.10 mg ai kernel^{-1} *B. firmus*). A greater rate of the biological component of the seed treatment may have resulted in differential RCA production compared to what was observed in this trail.

The increase in growth stage, height, and root area in the current study suggests treatment with clothianidin + *B. firmus* increased early-season growth in corn. These results are similar to those reported by Ding et al. (2018) where seed treatment with 1.0 or 2.0 mg ai kernel^{-1} clothianidin increased the height, growth index, and root length of seedlings in a field study in China. However, Wilde et al. (2007) observed no difference in root and shoot development with clothianadin and thiamethoxam seed treatment in a greenhouse study in Kansas. In 2000 and 2002, several locations experienced a decrease in days to half silk (2 of 3 in 2000 and 2 of 4 locations in 2002) when seeds were treated with a neonicotinoid seed insecticide (Wilde et al., 2004)suggesting possible accelerated phenological development as a result of applied seed treatment.

Conclusion

Treatment of corn seeds with clothianidin + *B. firmus* seed treatment did not promote RCA formation under the reduced stress environments. However, inclusion of the seed treatment did accelerate development even in the absence of insect pests. Future research should continue to assess how RCA may be impacted by seed treatment under adverse soil moisture conditions or altered concentrations of *B. firmus* as this may assist with crop tolerance to early-season abiotic stress.

Acknowledgements

Thank you to the Wilhelm and Eleanor Beckert Scholarship for funding this research, Bayer CropScience for donating the seed treatment materials, and Pioneer Hi-Bred International for the generous donation of two commercial hybrids.

Conflict of Interest

No conflict of interest is declared

References

Abendroth, L.J., R.W. Elmore, M.J. Boyer, and S.K. Marlay. 2011. Corn growth and development. PMR 1009. Iowa State Univ. Extension. Ames, IA.

AGDAILY Staff. 2017. Poncho/VOTiVO track record stretches across 40M acres. Retrieved from https://www.agdaily.com/crops/ponchovotivo-stretches-across-40m-acres/.

AgVenture. 2011. Introducing another tool in AgVenture Security Seed Protection System: Poncho 1250 VOTiVO.

Ding, J., Li, H., Zhang, Z., Lin, J., Liu, F., Mu, W. 2018. Thiamethoxam, Clothianidin, and Imidacloprid Seed Treatments Effectively Control Thrips on Corn Under Field Conditions. *Journal of Insect Science.* 18(6): 19. doi: 10.1093/jisesa/iey128

Drew, M.C., Morgan, P.W. 2000. Programmed cell death and aerenchyma formation in roots. *Trends in Plant Science.* 5(3):123-127.

Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R., Mearns, L.O. 2010. Climate Extremes: Observations, Modeling, and Impacts. *Science*. 289(5487): 2068-2074.

Heisey, P.W., and G.O. Edmeades. 1999. Maize production in drought-stressed environments: Technical options and research resource allocation. Part 1 of CIMMYT 1997/1998 World Facts and Trends; Maize Production in Drought-Stressed Environments: Technical Options and Research Resource Allocation. Mexico, D.F.: CIMMYT.

Karl, T.R., Knight, R.W., Easterling, D.R., Quayle, R.G. 1996. Indices of Climate Change for the United States. *Bulletin of the American Meteorological Society.* 77(2): 279-292.

Lenochova, Z., Soukup, A., Votrubova, O. 2009. Aerenchyma formation in maize roots. *Biologia Plantarum* 53(2):263-270.

Li, Y., K. Guan, G.D. Schnitkey, E. DeLuca, and B. Peng. 2019. Excessive rainfall leads to maize yield loss of a comparable magnitude to extreme drought in the United States. Glob. Change Biol. 2019:1-13.

Melillo, Jerry M., Terese (T.C.) Richmond, and Gary W. Yohe, Eds., 2014: Climate Change Impacts in the United States: The Third National Climate Assessment. U.S. Global Change Research Program, 841 pp. doi:10.7930/J0Z31WJ2.

Poncho VOTIVO Seed Treatment. 2019. Retrieved from https://agriculture.basf.us/crop-protection/products/poncho-votivo.html

Pujol, V., Wissuwa, M. 2018. Contrasting development of lysigenous aerenchyma in two rice genotypes under phosphorus deficiency. *BMC Res Notes*. 11(60). http://doi.org/10.1186/s13104-018-3179-y

Thomson, C.J., Armstrong, W., Waters, I., Greenway, H. 1990. Aerenchyma formation and associated oxygen movement in seminal and nodal roots of wheat. *Plant, Cell and Environment.* 13: 395-403.

Wilde, G., Roozeboom, K., Ahmad, A., Claassen, M., Gordon, B., Heer, W., Maddux, L., Martin, V., Evans, P., Kofoid, K., Long, J., Achlegel, A., Witt, M. 2007. Seed Treatment Effects on Early-Season Pests of Corn and on Corn Growth and Yield in the Absence of Insect Pests. *Journal of Agricultural and Urban Entomology*. 24(4): 177-193. https://doi.org/10.3954/1523-5475-24.4.177

Wilde, G., Roozeboom, K., Claassen, M., Janssen, K., Witt, M. 2004. Seed Treatment for Control of Early-Season Pests of Corn and Its Effect on Yield. Journal of Agricultural and *Urban Entomology.* 21(2): 75-85.

Wilson, M.J., Jackson, T.A. 2013. Progress in the commercialization of bionematicides. *BioControl.* 58(6):715-722.

Zhu, J., Brown, K.M., Lynch, J.P. 2010. Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays L.)*. *Plant, Cell and Environment*. 33: 740-749.